



TITLE:

Design, Synthesis and Applications of Selective  $\beta$ -Glycosidase Inhibitors,  $\beta$ -Glycosylamidines (MOLECULAR BIOFUNCTION - Chemistry of Molecular Biocatalysts)

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## Molecular Biofunction -Chemistry of Molecular Biocatalysts-



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### Visitors

Dr ZHANG Zhengzhu	Anhui Agricultural University, China, 1 October 2001 - 30 September 2002
Dr LUO Shaojun	Hanzhou Tea Research Institute, China, 2 October 2001 14 October 2001

## Scope of Research

Using various techniques of Natural Product Chemistry, Organic Synthetic Chemistry, Biochemistry as well as Molecular Biology, we are trying to clarify, on molecular basis, various biological events during life cycles where many kinds of biocatalysts (enzymes) are concerned. Our research covers the comprehensive understanding of the physiological roles of various kinds of biocatalysts and receptors as well as the reaction mechanism and specificity of each enzymatic reaction. **1)** Chemical, biochemical and molecular biological studies on  $\beta$ -primeverosidase (EC 3.2.1.149), a diglycosidase deeply concerned with tea aroma formation. **2)**

Design and synthesis of transition-state analogue and mechanism-based inhibitors of  $\gamma$ -glutamyltranspeptidase and  $\gamma$ -glutamylcysteine synthetase. **3)** Design and synthesis of glycosyl amidines as new glycosidase inhibitors and their application for affinity chromatography. **4)** X-Ray crystallographic analysis of pyruvate phosphate dikinase from maize. **5)** Directed evolutionary studies of *Pseudomonas* lipase. **6)** Molecular mechanism of the activation/inactivation process of plant hormones (brassinosteroides, gibberellins, cytokinins, *etc.*) by cytochrome P450. **7)** Molecular mechanism of plant resistance against disease infection specially interested in non-pathogenic *Fusarium* (NPF)-induced-resistance in sweet potato (*Ipomoea batatas* L.).

## Research Activities (Year 2001)

### Presentations

Presentations of each project (**1 - 7**) are as follows:

- 1)**  $\beta$ -Primeverosidase deeply concerned with floral tea aroma formation in oolong tea and black tea manufacturing, Sakata, K, Mizutani, M, Ma, S-J, Nakanishi, H, 11th World Congress of Food Science and Technology, Seoul, Korea, April 22-27, 2001 and **9 papers** in other meetings and symposia.
- 3)**  $\beta$ -Glycosylamidine as  $\beta$ -glycosidase

inhibitors with high potency and selectivity, Hiratake J, Kato M, Takada M (Nihon Shokuhin Kako Co., Ltd.), Inoue K, Yamamoto M (Nihon Shokuhin Kako Co., Ltd.), Sakata K, BioTrans 2001, Darmstadt, Germany, September 2-7, 2001 and **5 papers** in other meetings and symposia.

**4)** X-Ray crystallographic study on PPDK from maize, Nakanishi, T, Nakatsu, T (RIKEN), Matsumoto, T (RIKEN), Sakata, K, *et al.*, The 2001 Annual Meeting of

## Topics

### Design, Synthesis and Applications of Selective $\beta$ -Glycosidase Inhibitors, $\beta$ -Glycosylamidines

Selective inhibitors of glycosidases are of critical importance in developing chemotherapeutic agents and useful probes or tools to understand the function of glycosidases. We have developed highly selective  $\beta$ -glycosidase inhibitors,  $\beta$ -glycosylamidines **1a-c**, by incorporating the property of the transition state (a positive charge) into a chair-form sugar pyranose ring. The  $\beta$ -glycosylamidines were synthesized readily from the corresponding glycopyranoses in two steps without using protecting groups. The  $\beta$ -glycosylamidines **1a-c** inhibited the corresponding  $\beta$ -glycosidase with an inhibition constant ( $K_i$ ) of  $\mu\text{M}$  level, while they did not inhibit other glycosidases with different glycon- and stereospecificities; for example, no inhibition of  $\alpha$ -glucosidases,  $\alpha$ - and  $\beta$ -galactosidases and  $\beta$ -xylosidase by **1a**. The nature of the  $\beta$ -glycosylamidines as a “tailor-made” glycosidase inhibitor has been successfully used for affinity purification of glycosidases. Thus, the affinity adsorbents **2a** and **2b** were prepared with the  $\beta$ -glucosyl- and  $\beta$ -galactosylamidines as a ligand, respectively. A  $\beta$ -glucosidase from tea leaves was purified to almost homogeneity in one step from a crude enzyme extract by affinity chromatography using **2a** (Figure 1 and 2). Similarly, one-pot purification of a fungal  $\beta$ -galactosidase from crude enzyme preparation was also achieved by affinity chromatography on **2b**. The glycosylamidines thus promise “custom-made” preparation of glycosidase inhibitors and a useful tool for glycosidase study.

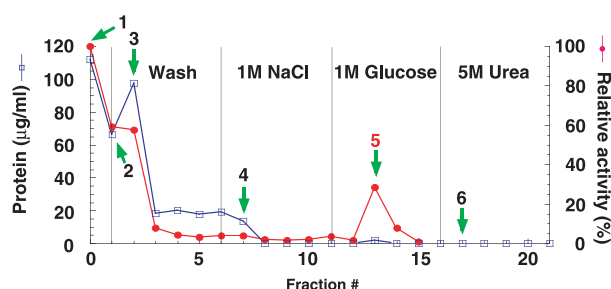
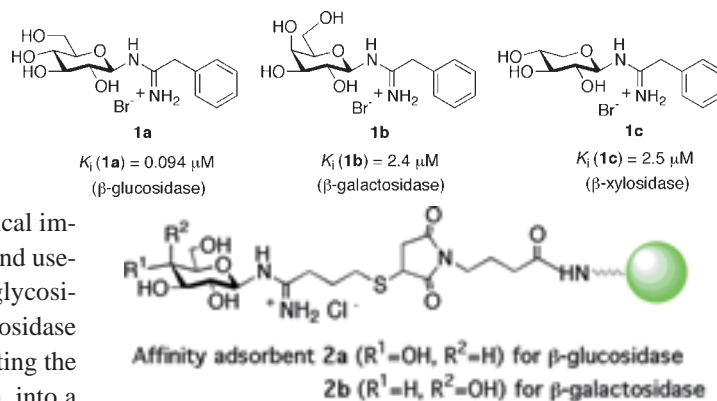


Figure 1. One Step Affinity Purification of  $\beta$ -Glucosidase from Tea Leaves.

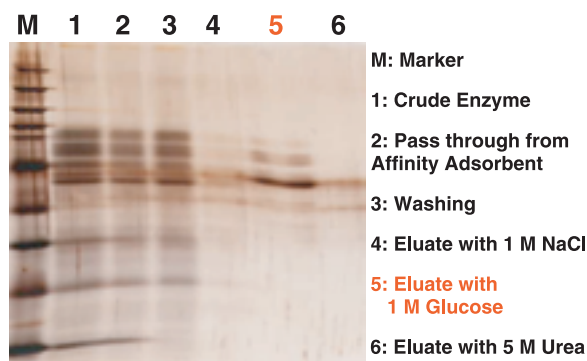


Figure 2. SDS-PAGE of Fractions Obtained by Affinity Chromatography.

Japanese Society for Bioscience, Biotechnology, and Agrochemistry, 24-27 March, Kyoto, 2001.

**5)** Random mutagenesis of lipase for amide hydrolyzing catalyst, Fujii R, Utunomiya Y, Atsushi S (Toyobo Co., Ltd.), *et al.*, BioTrans 2001, Darmstadt, Germany, September 2-7, 2001 and **3 papers** in other meetings and symposium.

**6)** Biochemical characterization of cytochrome P450s involved in brassinosteroid metabolism, Mizutani M, Ohta, D (Osaka Pref. Univ), Sakata K, 17th International Conference on Plant Growth Substances, Bruno, Czech Republic, July 4-5, 2001 and **11 papers** in other meetings and symposia.

And **5 presentations** on other research projects.

### Grants

Sakata K, Clarification of a new group of plant diglycosidase family, Grant-in-Aid for Scientific

Research (B) (2), 1 April 2001 - 31 March 2004.

Sakata K, A new combinatorial method to determine substrate specificities of glycosidases, Grant-in-Aid for Exploratory Research, 1 April 2001 - 31 March 2003.

Hiratake J, Bio- and organic chemical studies on glycosidases by using transition-state and substrate analogue inhibitors as a tool, Grant-in-Aid for Scientific Research (B) (2), 1 April 2001 - 31 March 2004.

Mizutani M, Molecular mechanisms of the activation /inactivation of a plant hormone, Grant-in-Aid for Scientific Research Priority Areas [(A) 13024243], 1 April 2001 - 31 March 2003.

Mizutani M, Investigation of the regulation mechanisms by which phytohormones control plant growth and development, Grant-in-Aid for Scientific Research from Kyoto University, 1 April 2001 - 31 March 2001.